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ORIGINAL PAPER

Lethal effects of pyrethrins on spruce budworm (*Choristoneura fumiferana*)

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Abstract: Spruce budworm (*Choristoneura fumiferana* (Clemens)) is one of the most serious forest insect pests in North America. Laboratory bioassays were performed to determine the lethal doses and lethal time of pyrethrins (a botanical insecticide) on 4th instar larvae of spruce budworm using larval dip assay. Results show that the LT_{50} values（time of 50% larval mortality）for spruce budworm at the pyrethrins concentrations of 12.5, 25, 50, 100, and 200 μg·L^{-1} were 94.78, 45.54, 20.36, 14.39 and 11.37 h, respectively. The percentage of cumulative mortality at the pyrethrins concentrations of 12.5, 25, 50, 100, 200 μg·L^{-1} was approximately 50%, 67%, 93%, 100% and 100% within 120 h, respectively. The LC_{50} value (concentration of 50% larval mortality) for the 4th instar larvae was 16.1μg·L^{-1}. Thus, larval mortality of spruce budworm increased in a concentration-dependent manner, and lethal time decreased with increasing pyrethin concentrations. These findings suggest that pyrethrins have a potential in controlling spruce budworm populations.

Key words: Spruce budworm (*Choristoneura fumiferana*); botanical insecticide; pyrethrins

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Introduction

The spruce budworm, *Choristoneura fumiferana* (Clemens), (Lepidoptera: Tortricidae), is one of the most destructive defoliators in the northeastern United States and eastern Canada (Cadogan et al. 2005). Its host plants include a variety of conifers (Talerico 1983), but spruce budworm prefers to feed on balsam fir (*Abies balsamea* [L.] Mill.), as well as spruce species (Cadogan et al. 2005). Chemical control was used to control spruce budworm outbreaks from 1950's to 1980's, such as synthetic chemicals of organophosphate, chlorinated hydrocarbons and carbamates (Nigam 1975; Shea and Nigam 1984).

The chemical pesticides can seriously threaten the endangered species, reduce biodiversity and induce the development of pesticide resistance and the resurgence of secondary pest populations. Thus, it is necessary to find the lower risk or environmentally-acceptable insecticides, based on entomopathogenic viruses, bacteria or fungi (EPA 2000). In particular, *Bacillus thuringiensis* subsp. *Kurstaki Berliner* (Btk) is one of the microbial insecticides that have been widely used successfully to control the spruce budworm in Canada since 1985 (Cadogan et al. 2004; Li et al. 2001). Although several types of viruses including nucleopolyhedrovirus (NPV), granulosis virus (GV), cytoplasmic polyhedrosis virus (CPV) and an entomopoxvirus (EPV) have been identified from spruce budworm populations (Cunningham 1985), field trails demonstrated that the efficacy of viruses was various (Cadogan et al. 2004). Hence, at present, the control of the spruce budworm over its range in North America is only dependent upon Bt-based insecticides. The repeated usage of Bt to control spruce budworm over a long time presents a great risk of pesticide resistance development on the spruce budworm. Thus, additional control options are practically needed.

The plant extracts such as rotenone from *Derris elliptica* (Ameen et al. 1983), azadirachtin from *Azadirachta indica* (Schmutterer 1981), capillin from *Artemisia nilagirica* (Banerji et al. 1990), arborine, a new bioactive compound related to quinazolone alkaloid, from *Glycosmis pentaphylla* (Muthukrish-

nan et al. 1999) and goniothalamin from *Bryonopsis laciniosa* (Kabir et al. 2003) have all been demonstrated to have insecticidal properties for a variety of insect pest species. In this study, pyrethrin insecticides derived from pyrethrum from the Chrysanthemum flower (*Chrysanthemum cinerariifolium*) (Klaassen et al. 1996) were selected for testing against spruce budworm larvae. This pyrethrin insecticide not only provides a quick knockdown of insects and breaks down very quickly in the environment, but also has low risk of residual environmental contamination and very low toxicity to mammals (Hitmi et al. 2000). A pyrethroid is a synthetic chemical compound similar to the natural pyrethrins. Pyrethroid tends to be more effective than natural pyrethrins (Klaassen et al. 1996) and usually more stable in the environment than pyrethrins (Elliott 1989). Therefore, in the study, the botanical insecticide (natural pyrethrin) was tested to determine its lethal effect on 4th instar spruce budworm larvae.

Materials and methods

Insects

Newly-hatched 1st spruce budworm instar larvae were purchased from the Canadian Forest Service Insect Production Unit, Sault Ste. Marie, Ontario. Larvae were reared on artificial diet in 30-mL plastic cups at room temperature (approximately 24ºC with 50%-60% R.H.). When larvae reached the 4th instar, they were selected as experimental insects.

Toxicity tests

Toxicity tests were conducted to determine the mortality of the budworm subjecting to pyrethrins 5.0 EC (Honghe senjun biology Co. LTD, P. R. China), containing 5% natural pyrethrum as the active ingredient. The pyrethrin 5.0 EC was diluted into six concentrations (200, 100, 50, 25, 12.5 and 6.25 μg·L^{-1}) by using distilled water (pH = 6.8) based on preliminary mortality tests. The results of preliminary mortality tests showed that approximately 50% mortality resulted from 12.5 μg·L^{-1} pyrethrin treatment, whereas about 95% mortality resulted from 50 μg·L^{-1} pyrethrin treatment.

For each concentration, 30 randomly-selected larvae were immersed in the insecticide solution or distilled water (control) for three seconds. After the larvae were blotted dry on filter paper, they were reared in cups containing artificial diet and held at room temperature. Five larvae were reared in each cup. Each treatment was replicated three times (a total of 90 larvae/treatment). Larval mortality was recorded at 6, 12, 24, 48, 72, 96 and 120 h after treatment. Larvae were considered dead if they did not respond when lightly prodded with forceps.

Data analysis

The mean percentage of larval mortality was normalized by an arcsine-square-root and then subjected to one-way analysis of variance (ANOVA), followed by Duncan's test to compare differences among the seven treatments at 0.05 significance level using DPS software (Tang and Feng 1997). However, only non-transformed means and variances are presented in Table1. The LC_{50} (concentration of 50% larval mortality) and LC_{95} (concentration of 95% larval mortality) were estimated according to the probit analysis. The data on larval mortality over time were analyzed with a complementary log-log model (CLL) (Preisler and Robertson 1989; Robertson and Preisler 1992). The LT_{50} (time of 50% larval mortality) and LT_{95} (time of 95% larval mortality) were estimated according to the methods of Preisler and Robertson (1989). The lethal-dose ratio test was used to determine the differences between LT_{50} and LT_{95} at $\alpha = 0.05$ level (Robertson and Preisler 1992) using DPS software.

Results

Effects of different pyrethrin concentrations on mortality of spruce budworm larvae

Within the first 6 h after treatment, no significant difference ($p > 0.05$) of mortality of spruce budworm larvae was found at the pyrethrin concentrations of 50, 100, and 200 μg·L^{-1} (Table 1). No larvae died at the pyrethrin concentrations of 0.00 and 6.25 μg·L^{-1} within 12 h (Table 1). From 12 h to 72 h, a significant increase ($p \leq 0.05$) in mortality was found with increasing pyrethrin concentrations (Table 1). Mortality had a rapid increase within 24 h in all treatments. The cumulative percentage of larval mortality slowed down and reached a stable maximum value by day 4 (96 h). No mortality was found in the control group. The mortalities at the pyrethrin concentrations of 6.25, 12.5, 25, 50, 100, 200 μg·L^{-1} were about 5%, 50%, 67%, 93%, 100% and 100% at 120h after treatment respectively (Table 1). Overall, there was a significant increase in mortality with increasing pyrethrin concentrations (Fig. 1). When pyrethrins were at the concentrations of 100 and 200 μg·L^{-1}, the 100% larval mortality occurred (Table 1). The rate of mortality at the pyrethrin concentration of 100 μg·L^{-1} was relatively slower than that that in the 200-μg·L^{-1} pyrethrin concentration (Fig. 1).

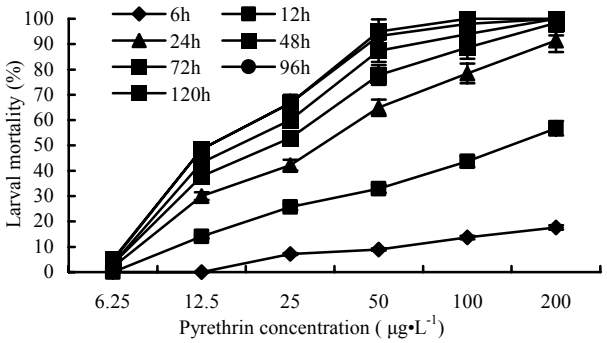

Fig. 1 Cumulative mortality of spruce budworm **larvae at 6, 12, 24, 48, 72, 96, and 120 h after treatments with different concentrations of pyrethrins 5.0 EC.** The concentrations were 6.25, 12.5, 25, 50, 100, 200 μg·L^{-1}. No larvae died in the control. The means of the three replicates ± SE are shown.

Table 1. Percentage of cumulative mortality of 4th instar spruce budworm larvae treated with different concentrations of pyrethrins 5.0 EC in a larval dip assay

Concentration ($\mu g \cdot L^{-1}$)	Larval morality±SE						
	6 h	12 h	24 h	48 h	72 h	96 h	120 h
0	0.00±0.00 c	0.00 ± 0.00 f	0.00 ± 0.00 g	0.00 ±0.00 g	0.00 ±0.00 g	0.00 ±0.00 f	0.00 ±0.00 f
6.25	0.00 ± 0.00 c	0.00 ± 0.00 f	2.22 ± 1.92 f	2.22 ±1.93 f	2.22 ±1.93 f	3.33 ±3.33 e	4.44 ± 1.93 e
12.5	0.00 ± 0.00c	15.56 ± 1.93 e	30.00 ±3.33 e	36.67 ±3.34 e	43.33 ±3.33 e	50.00 ±3.33 d	50.00 ± 3.32 d
25	6.67 ± 3.34 b	23.34 ± 3.33 d	43.33 ±3.33 d	53.33 ±3.11 d	60.00 ±3.33 d	66.67 ±3.34 c	66.67 ± 3.34 c
50	10.00 ± 3.33 ab	30.00 ± 3.33 c	65.56 ±5.09 c	76.67 ±3.34 c	86.67 ±3.34 c	93.33 ±3.34 b	93.33 ± 3.34 b
100	13.33 ± 3.34 a	43.33 ± 3.34 b	78.86 ±5.10 b	90.00 ±3.21 b	92.22 ±1.93 b	97.78 ±1.91 a	100.00 ± 0.00 a
200	13.34 ± 3.34 a	56.67 ± 3.22 a	88.89 ±1.93 a	97.78 ±1.93 a	100.00 ±0.00 a	100.00±0.00 a	100.00 ± 0.00 a

Notes: 4th instar spruce budworm larvae (three replicates of 30 larvae per replicate) were tested using larval dip assay. The mortality was recorded on 6, 12, 24, 48, 72, 96, 120 h post-dip. The means of the three replicates ± SE are shown. Means within columns followed by different letters were significantly different based on Duncan's multiple range test ($p \leq 0.05$).

Lethal dose and lethal time for spruce budworm larvae treated with pyrethrins

The LC_{50} was decreased with the time and was estimated as 23.91$\mu g \cdot L^{-1}$ and 16.11 $\mu g \cdot L^{-1}$ in 48h and 120h, respectively (Table 2). The lethal time varied in a concentration – dependent manner. The LT_{50} values for spruce budworm at the pyrethrin concentration of 12.5, 25, 50, 100, and 200 $\mu g \cdot L^{-1}$ were estimated as 94.78, 45.54, 20.36, 14.39, and 11.37 h, respectively (Table 3). The LT_{95} values at the pyrethrin concentration of 50, 100 and 200 $\mu g \cdot L^{-1}$ were 119.71, 64.50 and 31.78 h, respectively (Table 3). Higher pyrethrin concentrations may cause larvae to die within shorter time periods (Table 3). The lethal-dose ratio test shows that there was a significant difference ($p \leq 0.05$) in LT_{50} between all pyrethrin concentrations, except for the concentration of 200 vs. 100 $\mu g \cdot L^{-1}$ (Table 4). LT_{95} ratio (95%CL) significantly increased with decreasing pyrethrin concentration except for the pyrethrin concentration of 12.50 vs. 25$\mu g \cdot L^{-1}$ (Table 4). Thus, larval mortality of spruce budworm was increased in a concentration-dependent manner, and lethal time was decreased with increasing pyrethin concentrations.

Table 2. Lethal concentrations for spruce budworm treated with different concentrations of pyrethrins 5.0 EC using larval-dip assay

Time (h)	Slope ± SE	LC_{50}(95%CL) ($\mu g \cdot L^{-1}$)	x^2	d*f*
6	0.92 ± 0.33	1978.84(341.61-535.48)	2.50	4
12	1.22 ± 0.23	131.67(80.24-323.84)	2.98	4
24	1.79± 0.24	33.62(25.29-44.33)	3.70	4
48	2.27 ± 0.28	23.91(17.96-30.24)	3.96	4
72	2.55 ± 0.33	19.82(14.48-25.05)	6.34	4
96	3.02 ± 0.41	16.54(11.56-21.08)	5.34	4
120	3.23± 0.44	16.11(11.17-20.51)	4.45	4

Notes: 4th instar spruce budworm larvae (three replicates of 30 larvae per replicate) were tested using larval dip assay. The mortality was recorded on 6, 12, 24, 48, 72, 96, 120h post-dip. The means of the three replicates ± SE are shown. Each value of slope represented the regression slope of the relationship between larval mortality and lethal concentration.

Table 3. Lethal time for spruce budworm treated with different concentrations of pyrethrins 5.0 EC using larval dip assay

Pyrethrins ($\mu g \cdot L^{-1}$)	Slope±SE	LT_{50}(95%CL) (h)	LT_{95}(95%CL) (h)	x^2	d*f*
12.5	1.29±0.24	94.78 (63.70-191.70)	1770.89 (595.97-17793.5)	3.72	5
25	1.35±0.22	45.54 (33.38-65.89)	761.73 (343.83-3433.42)	2.01	5
50	2.14±0.26	20.36 (15.13-25.73)	119.71 (88.05-187.02)	1.67	5
100	2.52±0.30	14.39 (9.97-18.67)	64.50 (51.24-87.18)	2.70	5
200	3.69±0.54	11.37 (6.86-15.36)	31.78 (25.99-38.11)	0.68	5

Notes: 4th instar spruce budworm larvae (three replicates of 30 larvae per replicate) were tested using larval dip assay. The mortality was recorded at 6, 12, 24, 48, 72, 96, 120h post-dip. The means of the three replicates ± SE are shown. Each value of slope represented the regression slope of the relationship between larval mortality and lethal time.

Discussion

The mortality of spruce budworm was higher after exposured to low concentrations of the pyrethroid and resmethrin (Morris 1975). However, the synthetic pyrethroid insecticides have disadvantages, including insecticide residues and potential human heath risks. To mitigate these risks, pyrethroid insecticides were restricted from use in some areas. In 2006, EPA (Environmental protection agency in USA) announced the permethrin registration eligibility decision including application rates, droplet size and mandatory label statements.

In our preliminary study, higher mortality of spruce budworm was also found in all pyrethrin treatments in comparison with the control and the capsaicin-nicotine (botanical insecticide). Our results demonstrated that natural pyrethrins caused considerable mortality to larvae of the spruce budworm. Similar results were found in privious reports, demonstrating that pyrethrins have a considerable lethal effect on other insect species, such as leaf-

hopper (*Empoasca fabae*) (Maletta et al. 2006) and grape root borer (*Vitacea polistiformis*) (Weihman and Liburd 2006*)*. In our study, the LC_{50} of pyrethrins for spruce budworm was estimated as 16.11 μg·L^{-1}. According to the study of Sheppard and Brad (2000), LC_{50} of pyrethrin I and pyrethrin II for house flies (*Musca domestica*) was 0.20 μg·L^{-1} and 0.49 μg·L^{-1}, respectively.

Table 4. Comparisons of lethal time for spruce budworm treated with different concentration of pyrethrins 5.0 EC using larval dip assay

Pyrethrins (μg·L^{-1})	LT_{50} ratio (95%CL)				
	12.5	25	50	100	200
12.5	1.00	2.08*(1.23-3.53)	4.66*(2.87-7.58)	6.59*(4.07-10.67)	8.34*(5.25-13.24)
25	0.50*(0.30-0.84)	1.00	2.24*(1.51-3.31)	3.17*(2.15-4.67)	4.01*(2.79-5.76)
50	0.21*(0.13-0.35)	0.43*(0.30-0.61)	1.00	1.42*(1.02-1.97)	1.79*(1.33-2.42)
100	0.15*(0.09-0.25)	0.30*(0.21-0.43)	0.71*(0.51-0.98)	1.00	1.27(0.94-1.70)
200	0.12*(0.08-0.19)	0.24*(0.17-0.33)	0.56*(0.41-0.75)	0.79(0.59-1.06)	1.00
Pyrethrins (μg·L^{-1})	LT_{95} ratio(95%CL)				
	12.5	25	50	100	200
12.5	1.00	2.33 (0.42-12.82)	14.85*(3.53-62.53)	56.06*(6.64-114.55)	56.06*(13.58-231.47)
25	0.31(0.06-1.48)	1.00	6.38*(2.17-18.76)	11.86*(4.11-34.20)	24.11*(8.43-68.97)
50	0.07*(0.02-0.28)	0.22*(0.09-0.51)	1.00	1.86*(1.10-3.14)	3.78*(2.27-6.28)
100	0.04*(0.01-0.15)	0.12*(0.05-0.27)	0.54*(0.32-0.91)	1.00	2.03*(1.27-3.25)
200	0.02*(0.01-0.07)	0.06*(0.03-0.13)	0.26*(0.16-0.44)	0.49*(0.31-0.79)	1.00

Notes: An asterisk (*) indicates a significant ($p \leq 0.05$) difference in LT_{50} and LT_{95} values between any concentrations of pyrethrins based on the 95% confidence limits (CL) of the ratio excluding 1 (Robertson and Preisler, 1992).

Benezet et al. (1988) found that the toxicity of the pyrethrins decreased with increasing temperature. As expected, there was a significant increase in mortality of spruce budworm with increasing concentrations from 12.5 to 200 μg·L^{-1} from 24h to 96h. This result was in agreement with results of Barcic et al. (2006). Therefore, the spruce budworm larval mortality varied in a concentration-dependent manner. That is to say, the spruce budworm larval morality is associated with time.

Previous studies have demonstrated that lethal time decreases with increasing concentrations of active ingredient (*e.g.* Li et al. 2003; Poland et al. 2006). Similarly, in our study, the lethal time decreased from approximately 120 h to 95 h with increasing of the concentration of pyrethrins. Appel et al. (2004) found that the LT_{50} value of Drione (silica gel and synergized pyrethrins) decreased with increasing concentration and relative humidity in a study of the toxicity of Drione insecticidal against German cockroaches (*Blattella germanica*). Therefore, suitable humidity condition might accelerate the efficacy of pyrethrins at the same concentration, which needs further study.

This botanical insecticide is naturally derived from plants with low toxicity to mammals and fast biodegradation rate. Also, it has very low possible impact on development of insecticide resistance (Barcic et al. 2006). Based on our results, applications of low-dose (i.e., 50 μg·L^{-1}) pyrethrins could provide effective control for larval spruce budworm. Zapata et al. (2006) reported that the natural pyrethrins compounded piperonil butoxide (PBO) (LC_{50} =50.6 μg·L^{-1} in 72 h) reduced the population of Mediterranean fruit fly (*Ceratitis capitata*). Igrc Barcic et al. (2006) reported that the application of pyrethrin with low dose (170 mL·ha^{-1}) significantly reduced leaf damage from the Colorado potato beetle (*Leptinotarsa decemlineata*). Therefore, pyrethrins may have potential to be used in controlling spruce budworm populations.

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